The Harlequin ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae) in the Western Cape Province, South Africa: effects on arthropods in urban, agricultural and natural ecosystems

by
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Declaration

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Abstract

Invasive arthropod predators are one of the largest and most diverse groups of invasive insects in the world. Many are generalist predators, with cosmopolitan distributions due to their use as biological control agents in agriculture. *Harmonia axyridis* (Coleoptera: Coccinellidae), an invasive arthropod predator species native to Asia, which now has a world-wide distribution. It is considered one of the most successful biological control predator species and is generally considered to be economically beneficial. However, negative effects have recently emerged in agricultural and natural systems. *Harmonia axyridis* poses a threat to biodiversity as it outcompetes native species for food resources. It can also feed directly on native predatory arthropods that disrupt natural ecosystem processes. Their movement in-and-out of agricultural landscapes may depend on food availability with natural vegetation alongside agricultural areas often utilised for refuge and alternative food resources. This beetle has also been recorded in urban areas. The aim of this study was to determine how the invasive *H. axyridis* beetle uses the local landscape in the Western Cape province, South Africa, and to determine its threat to native species. I sampled urban landscapes, vineyards, natural vegetation/vineyard edge zones and pristine natural areas for arthropods every second month using a suction sampler. Data collected included the abundance and diversity of *H. axyridis*, herbivores, local predators and non-*Harmonia* ladybeetles. Most *H. axyridis* were collected in urban areas during all sampling periods. Highest abundance was recorded in May and July (winter). This indicates that urban areas were the preferred landscape feature and that these act as ovipositing areas, particularly as larval *H. axyridis* were also only collected in urban areas. Significantly, vineyards and natural vegetation had very low abundance of *H. axyridis*, questioning their value as a biological control agent in this region. *Harmonia axyridis* had a negative effect on the overall local arthropod community, as well as the predator and herbivore guilds, although it was positively correlated with the abundance of non-*Harmonia* ladybeetles. This suggests that *H. axyridis* and non-*Harmonia* ladybeetles are responding to the same resources in these landscapes. A negative correlation found between *H. axyridis* and the abundance of predators is most likely due to competition for the same resources (e.g. prey items). These negative impacts, along with their negligible value as biological

control agents in agriculture, suggest that a programme should be implemented to control this invasive species. More specifically, control should be aimed in urban areas during winter when and where the species aggregates and when larvae are present.

Opsomming

Geleedpotige roofdiere is een van die grootste en mees diverse groepe van uitheemse insekte in die wêreld. Die meeste is veelsydige roofdiere, met wêreldwye verspreiding te danke aan hul gebruik as biologiese beheer agente in landbou gebiede. Byvoorbeeld, *Harmonia axyridis* (Coleoptera: Coccinellidae), 'n indringer geleedpotige roofdier spesies inheems aan Asië, het nou 'n wêreldwye verspreiding. Dit word beskou as die mees suksesvolle roofdier spesies wat gebruik word vir biologiese beheer en word oor die algemeen beskou as ekonomies voordelig. Negatiewe effekte was onlangs aangeteken beide in landbou gebiede en natuurlike areas. *Harmonia axyridis* hou 'n bedreiging in vir inheemse biodiversiteit as dit inheemse spesies uitkompeteer vir voedsel bronne. Dit kan ook direk voed op plaaslike roofsugtige geleedpotiges wat trofiese vlakke ontwrig en uiteindelik, biodiversiteit. Hulle beweging in-en-uit landbou landskappe kan gekoppel word aan die beskikbaarheid van voedsel, en gebruik natuurlike plantegroei langs landbou gebiede dikwels as 'n toevlugs oord en area vir alternatiewe voedsel bronne. *Harmonia axyridis* word ook in stedelike gebiede aangeteken. Die doel van hierdie studie was om te bepaal hoe die indringer Harlekynkewer die plaaslike landskap gebruik met die fokus op wingerde in die Wes-Kaap provinsie van Suid-Afrika, en tweedens om die bedreiging wat hierdie kewer moontlik vir inheemse spesies te bepaal. Ek het arthropoda in stedelike landskappe, wingerde, natuurlike plantegroei / wingerd rand sones en ongerepte natuurlike areas elke twee maande met behulp van 'n D-vac versamel. Monsters was ontleed deur gebruik te maak van die getalle van *H. axyridis*, herbivore, plaaslike roofdiere en nie-*Harmonia* liewenheers kewers. Die meeste *H. axyridis* was in stedelike gebiede versamel gedurende al die seisoene, maar meeste individue was gedurende Mei en Julie (winter) versamel. Hierdie toon dat stedelike gebiede die voorkeur-landskappe is vir hierdie kewers en dat hierdie gebiede opgetree as eierleggende gebiede, veral omdat larwes van *H. axyridis* slegs in hierdie gebiede aangeteken was. Wingerde en die natuurlike plantegroei het baie lae getalle *H. axyridis* gehuisves wat hul waarde as biobeheermiddel bevraagteken. *Harmonia axyridis* het 'n negatiewe uitwerking op die algehele plaaslike geleedpotige gemeenskappe gehad, asook op die die roofdier en herbivoor gildes, maar hul getalle was positief gekorreleer met die getalle van nie-*Harmonia* liewenheerskewers. Dit dui daarop

dat *H. axyridis* en nie-*Harmonia* liewenheerskewers beide reageer op dieselfde hulpbronne in hierdie landskappe. 'n Negatiewe korrelasie was gevind tussen die getalle van *H. axyridis* en die getalle van ander predatoriese geleedpotiges at waarskynlik te danke was aan mededinging tussen hierdie groepe vir dieselfde hulpbronne (bv prooi). Hierdie negatiewe invloede, asook hul verminderde waarde as biobeheeragente in die landbou, dui daarop dat 'n program in werking gestel moet word om hierdie indringerspesies te beheer. Meer spesifiek, beheer moet gedurende die winter en in stedelike gebiede geskied, waar en wanneer hierdie spesie op sy volopste is en waar larwes teenwoordig is.

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Chapter 1: Introduction

1.1. Agricultural landscapes

An ever increasing human population has led to a higher demand for food from agriculture, resulting in a constantly expanding agricultural industry (Tilman et al., 2002; Matson and Vitousek, 2006). For example, between 1980 and 2001, agricultural lands have increased by 35% in developing tropical countries and are still continuing today (Medley et al., 1995; Abdullah and Nakagoshi, 2008). This conflict between land for nature and land for agriculture has intensified recently due to a scarcity of available land (Heitala-Koivu, 1999). The increase in land transformation for agriculture reduces natural vegetation and negatively affects natural biodiversity and normal ecosystem function (Matson et al., 1997). Agricultural landscapes are spatially heterogeneous, with different agricultural crops adding to different land cover types, with diverse impacts on native systems (Fahrig et al, 2011). For example, numerous diverse activities have been introduced to increase production of various crops, such as the use of different chemicals to regulate various pests and weeds, each requiring different management regimes (Medley et al., 1995; Meehan et al., 2011).

Yet, increase in agriculture is vital to maintain human existence as human population levels are expected to exceed earth's carrying capacity. The process of habitat fragmentation in agricultural landscapes is a major threat to native biodiversity and has led to many species going extinct (Grashof-Bokdam, 1997). One of the most important scientific challenges now is to find a way to retain biodiversity and maintain natural ecosystem function in production landscapes (Samways et al., 2010). In production landscapes, most of the remaining natural vegetation is in isolated patches (Tscharntke et al., 2005) while the rest of the area has been ecologically simplified into monoculture stands (Meehan et al., 2011). However, conserving natural biodiversity in these small natural patches remains a daunting task as processes influencing organisms in these are still not clearly understood (Zuidema et al., 1996; Fischer and Lindenmayer, 2002). Fragmentation also affects the movement of organisms negatively (Eigenbrod et al., 2008) and impacts on organism adaptation to changing environments (Andren, 1994). However, compared to other land transformation pressures such as

urbanisation (Rebello et al., 2011), agroecosystems are still regarded as fairly rich in biodiversity (Perfecto et al., 1997). Most of this diversity is contained in the natural fragments (Altieri, 1999). This remnant biodiversity can be very beneficial to producers, as these provide ecosystem services such as nutrient cycling, pollination, regulation of microclimates, suppression of pests and positively impact on hydrological processes (Altieri, 1999).

1.2. Cape Floristic Region

The Cape Floristic Region (CFR) of the Western Cape Province, South Africa, is situated in a high intensity agricultural area (Fairbanks et al., 2004). The CFR is situated at the southwestern part of Africa (Goldblatt and Manning, 2002), and is considered as one of the world's smallest floral kingdoms with an area of only about 90 000 km^2. This region, with its Mediterranean climate with cold wet winters and hot dry summers (Goldblatt, 1997), is known for its high plant species richness and diversity, as well as high levels of endemism (Helme and Tinder-Smith, 2006). It comprises the Fynbos biome, Succulent Karoo biome, Thicket biome and Afrotemperate forest biome (Mucina and Rutherford, 2006). Furthermore, the Fynbos biome is characterised by different vegetation types, namely fynbos, renosterveld and strandveld (Mucina and Rutherford, 2006).

The CFR has been irreversibly transformed by agriculture, alien plant invasions and urban development (Richardson et al., 1996; Rouget et al., 2003; Latimer et al., 2004). Like in many parts of the world, agriculture is one of the leading causes of landscape transformation in the CFR, where much of the fynbos biome has been transformed into agricultural lands (McDowell and Moll, 1992; Kemper et al., 2000), and with the expansion of the wine industry in Western Cape, expectations are that more land transformation will take place (Fairbanks et al., 2004).

Agriculture and invasion by non-native species has become the leading threats to the Cape floristic biodiversity (Holmes and Cowling, 1997; Witt and Samways, 2004). Ten vegetation types are classified as Critically Endangered ecosystems in the CFR, where four are Endangered and another four Vulnerable. Only five are classified as Least Threatened (Rebello et al., 2011). Urbanised areas

in the CFR contributes 42% of the 24 South African Critically Endangered vegetation types identified in 2004, and 52% of the 21 current Critically Endangered vegetation types. Six vegetation types are endemic to Metropolitan Cape Town, with three of these Critically Endangered and the remainder classified as Endangered (Rebello et al., 2011). From all these vegetation types, Alluvium Fynbos is the most transformed with only 6% remaining and hereafter Renosterveld with only ca. 30% remaining.

1.3. Arthropods in the agricultural landscape

Native arthropods persist in remaining patches of natural vegetation in production landscapes. Natural habitats support a large number of arthropods as they provide shelter and alternative food resources (Stamps and Linit, 1998). The relatively high plant diversity in natural vegetation supports a high diversity of arthropods due to increasing habitat structural complexity (Tews et al., 2004). However, factors such as patch size, patch isolation, patch density, landscape structure and landscape composition can influence the occurrence, abundance and distribution of arthropods in agricultural landscapes (Hunter, 2002).

Crop fields are subjected to change e.g. when they are cleared after harvesting and the periodic use of chemicals to regulate pests (Obrycki and Kring, 1998). This leads to loss of biodiversity in crop fields, with many living organisms moving to adjacent natural habitats (Thomson and Hoffman, 2009). When crops become available again in the next growing season they move back to the fields (O'Neil and Wiedenmann, 1987). Therefore, pests often inhabit both agricultural and natural habitats, as they move in and out between the two habitats in search of food and shelter (Bianchi et al., 2006; Johnson and Beck, 1988, Thomson and Hoffman, 2009; Thomson and Hoffman, 2010; Thomson et al., 2010). Many predators also use both natural habitats and agricultural fields in the search of food and can help reduce pest numbers in both (O'Neil and Wiedenmann, 1987). Without disturbances such as pesticide use in the crop fields, crops can actually provide good habitat and food resources for numerous arthropods within and around these fields (Tscharnkte et al., 2005).

Arthropods provide ecosystem services to agricultural areas such as pollination and biological control of pests and weeds (Gardiner et al., 2009; Losey and Vaughan, 2006). Their diversity in agricultural landscapes are however greatly influenced by the structure and composition of the natural vegetation remnants in these areas (Marino and Landis, 1996; Colunga-Garcia et al., 1997). This is because natural vegetation often provides alternative food resources (e.g. pollen and nectar, prey), overwintering sites and/or refugia for these important groups (Lykouressis et al., 2008; Landis et al., 2000). Therefore, the disturbance of natural plant diversity can disrupt these arthropods communities and can lead to a decline in the numbers of many of these beneficial species (Letourneau et al., 2011).

1.4. Invasive species

Invasive species are non-indigenous species that disperse and integrate in ecosystems far beyond areas where they have been introduced (Richardson et al., 2000). Most invasive species are long lived, aggressive and undergo rapid population growth (Sujay et al., 2010). Most invasive species have been distributed throughout the world through transportation of goods by humans (Holmes et al., 2009; Hulme, 2009). Alarmingly, many of these species are introduced in new areas for the purpose of biological control, with devastating consequences for natural biodiversity (van Wilgen and de Lange, 2011). For example, parasitoids introduced in Hawaii between 1913 and 1950 to control three fruit flies (*Ceratitis capitata*, *Bactrocera dorsalis*, *B. curcurbitae*) were successful in biological control, but negative effects on non-targets species were recorded after they spread to natural habitats (Louda et al., 2003).

Invasive species are some of the biggest contributors to ecosystem change and biodiversity loss globally (Pysek and Richardson, 2010). They have the ability to change the function and structure of ecosystems (Ehrenfeld, 2010; van Wilgen and de Lange, 2011; Simberloff et al., 2013) and to compete with native species for resources (Pimentel et al., 2001). In severe cases, invasive species can lead to the extinction of native species (Gurevitch and Padilla, 2004). From an agricultural viewpoint, invasive species can cause economic loss when they disrupt ecosystem services provided by native

species (Pimentel et al., 2005; Cook et al., 2007). Some have the ability to change geomorphological processes and biogeochemical or hydrological cycling in ecosystems (Gordon, 1998). Others can change the composition and function of microbial communities, structure of food webs and nutrient cycling (Ratahiriarisoa et al., 2015). For example, *Casuarina equisetifolia* (Casuarinaceae) is an invasive plant species from Australia, Melanesia and Southeast Asia, it has been planted in many countries around the world where it replaces native species and depletes essential soil nutrients such as nitrogen (Ratahiriarisoa et al., 2015). *Melaleuca quinquenervia* (Myrtaceae) another invasive plant species from Australia, was introduced in Florida USA in 1900 as an ornamental. It transforms wetlands areas into forest (Turner et al., 1998). *Nassella trichotoma* (Poaceae) is an invasive perennial grass introduced in Australia from South America in the 1900s. Today it is found across southern Australia. This invasive grass has no grazing value, reduces livestock carrying capacity, decreases biodiversity in native grasslands, reduces land value and is a fire hazard (Klepeis et al., 2009).

1.5. Invasive arthropods

Invasive arthropods are one of the largest and most diverse groups of invasive alien species in the world (Roy et al., 2011; Engelkes and Mills, 2011). It is important to note that both intentionally and accidentally introduced invasive species are capable of causing a threat to native biodiversity (Engelkes and Mills, 2011). As an example, many of these invasive arthropods are predators, parasitoids or herbivores that are used in biological control and have been introduced in agricultural areas intentionally (Colunga-Garcia and Gage, 1998; Hoddle, 2004). As a result, agricultural and plantation areas experience high establishment of invasive species, as more introductions are done in these areas (Picker and Griffiths, 2011). However, due to the high dispersal rate of invasive arthropods, they invade non-target native habitats (Picker and Griffiths, 2011). In extreme cases, some invasive arthropods move to native species as a preferred host (Hartley et al., 2010).

Many invasive arthropod species are generalist feeders and play a significant role in pest reductions (Snyder and Evans, 2006). However, successful introductions of generalist predators as biological

control agents are often associated with negative impacts on native communities due to their broad diet which includes non-target species (Snyder and Ives, 2003). In most countries, the arrival of these species has affected the native biodiversity negatively (Kindlmann et al., 2011). They are capable of invading and disrupting normal ecosystem functioning through competition and direct attack and consumption of other organisms (Elliott et al., 1996). For example, *Compsilura concinnata* (Tachinidae) was introduced in North America in 1906 to control the gypsy moth (*Lymantria dispar*) and the browntail moth (*Euproctis chrysorrhoea*). It is a multivoltine species and preferred other native species including Lepidoptera and Hymenoptera (Louda et al., 2003). *Cotesia glomerata* (Braconidae) was released in North America in 1880s to control an invasive pest, *Pieris rapae* (Pieridae). However, this control agent attacks many native species such as *P. napi oleracea* in northeastern United States and eastern Canada (Louda et al., 2003). *Meteorus laphygmae* (Braconidae) and *Cotesia marginiventris* (Braconidae) were released in Hawai'i in 1942 to control lepidopteran pests. They quickly became dominant and attacked many native Lepidoptera (Louda et al., 2003).

Many invasive herbivores also have a wide distribution as they can be introduced as biological control agents or by accidental introduction during transportation of goods (Simberloff and Stiling, 1996). Some of these herbivores are capable of expanding their host ranges to native plants and may also have indirect negative effects through competition with native herbivores (Henneman and Memmott, 2001). For example, *Rhinocyllus conicus* (Curculionidae) native to Eurasia was released in Argentina, Australia, New Zealand, and North America to control weedy thistles (Asteraceae, Carduinae) such as *Carduus nutans*. This species invaded other habitats with native thistles where it causes reduced seed production (Louda et al., 2003). *Larinus planus* (Curculionidae) was released in United States (Colorado) in 1990s to control Canada thistle. It was recorded feeding on native thistle (*Cirsium undulatum*) in western Colorado where it destroyed its seed-producing flowers (Louda et al., 2003).

Accidentally introduced invasive herbivores have also done great damage in both agricultural areas and natural habitats (Gandhi and Herms, 2010) around the world (Paini et al., 2010). For example, *Chilo partellus* (Crambidae), native to Asia, cause damage to agricultural crops such as sorghum and

sugarcane (Annecke and Moran, 1982). *Myzus persicae* (Aphididae) native to Asia, is now distributed throughout the world and is a serious pest of many agricultural crops, and can transmit plant viruses (Blackman and Eastop, 2000).

Invasion by pollinators may also have a negative impact on native plant-pollinator interactions (Vila et al., 2009). Invasive pollinators can outcompete native pollinators, leading to a decline in the services that these provide (Traveset and Richardson, 2006). For example, bumblebees (*Bombus terrestris*), originally from Europe, are now widely distributed across the world (Inoue et al., 2008). They have been widely used as pollinators of greenhouse crops, but in many areas have escaped these closed systems. They compete with native fauna and decreases pollination success of many native plants (Inoue et al., 2008).

Even exotic detrivores can have severe negative impacts on native ecosystems. These organisms often play a significant role in the ecosystems such as decomposing of leaves that reduces accumulation and ultimately flammability, they help bind sub-surface organics, can reduce soil erosion, recycle nutrients, and reduce the surface tension of soil particles that can help soils to retain moisture (Sands and Goolsby, 2011). However, exotic species can compete with native species for resources such as floor organic litter and can reduce the abundance of native species with negative effects on these processes (Sands and Goolsby, 2011). For example, various saw bugs have been transported around the world via logs, pot plants and other goods (Barnard, 1932). They can negatively influence native ecosystems by competing with native species for resources such as floor organic litter thereby reducing the abundance of native species (Sands and Goolsby, 2011).

1.6. South African invasive arthropods

Many invasive arthropod species have been recorded in South Africa (Picker and Griffiths, 2011). Many are considered to have arrived in South Africa through importation of goods while others were introduced for biological control (Giliomee, 2011). However, very few of these invasive arthropods have been studied to clarify their impacts to native biodiversity (Picker and Griffiths, 2011). Most

accidentally introduced invasive arthropods have arrived in South Africa more than 100 years ago, whereas intentional introduction for biological control only started recently (Picker and Griffiths, 2011). Luckily, only a few have managed to colonise natural areas and cause damage (Picker and Griffiths, 2011). For example, the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae) is one of the most invasive insects globally, which was accidentally introduced through boat transportation from its origin in Argentina to other parts of the world, including South Africa (Heller et al., 2006). The Argentine ant has been reported to displace native species (Witt and Giliomee, 2004; Witt, 2006) with negative effects on native plants in the CFR that rely on native ants for seed dispersal (Bond and Slingsby, 1984).

Economically speaking however, many negative effects have been recorded. For example, codling moth, *Cydia pomonella*, native to temperate Asia, is regarded as one of the most economically important and most widely distributed pest species (Ii'chev, 2004). It is a pest of apples and many other plants, where it causes damage on fruit (Annecke and Moran, 1982). *Vespula germanica* (Vespidae) is an invasive wasp from Europe that was accidentally introduced in South Africa (Tribe and Richardson, 1994; Allsopp and Tribe, 2003). This invasive species is known to have negative effects in the agricultural sector as it damages fruits, and to the native fauna through competition for food resources (Allsopp and Tribe, 2003). *Sirex noctilio* (Hymenoptera: Siricidae) native to temperate Eurasia was also accidentally introduced in South Africa through importation of timber, and now it is a pest in pine plantations (*Pinus radiata*) (Tribe and Cillié, 2004). *Caliroa cerasi* (Tenthredinidae) was accidentally introduced in South Africa from Europe or Asia where it now damages fruit (Kaiser and Shread, 2001).

There are a number of insects that were intentionally introduced in South Africa. For example, *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) was introduced to South Africa from Australia to control an invasive plant species *Acacia longifolia* also an Australian native (Dennill et al., 1993; Prinloo and Neser, 2007). *Cotesia plutellae* (Braconidae) was deliberately introduced in South Africa from Europe as biological control agent to control Diamondback moth (Nofemela and Kfir, 2005; Safraz et al., 2005). *Apanteles subandinus* (Braconidae) was also introduced as a

biological agent to control potato tuber moth which is from South America (Watmough et al., 1973; Neuenshwander et al., 2003). *Megalyra fasciipennis* (Megalyridae) was intentionally introduced in South Africa from Australia as biological control agent to control invasive pests beetles (Gess, 1964). These are good examples of success stories, but some intentionally introduced species may also have negative effects on native biota. E.g. *Stenopelmus rufinasus* (Curculionidae) native to the Americas was intentionally introduced in South Africa to control the red water fern (*Azolla filiculoides*) in aquatic ecosystem (Hill, 1999). This beetle is considered as one of the most successful biological control agent of weeds, after successfully controlling *Azolla filiculoides* weed (Hill, 1999). However, negative effects have been recorded from this beetle as it caused the extinction of the native red water fern (McConnachie et al., 2003). *Leptinotarsa texana* and *L. defecta* (Chrysomelidae) also native to the Americas were intentionally introduced in South Africa to control weeds (Olckers et al., 1999). They successfully control *Solanum elaeagnifolium* (Olckers et al., 1999), but also attack native plants, especially when *Solanum elaeagnifolium* is no longer available (Hoffman et al., 1998).

1.7. The invasive Harlequin beetle (*Harmonia axyridis*)

Coccinellids include some of the most invasive arthropod predators in the world as they are widely transported for use as biological control agents (Obrycki and Kring, 1998). *Harmonia axyridis* (Coleoptera: Coccinellidae) is an invasive arthropod predator native to Asia (Koch, 2003) that has been repeatedly introduced in agricultural areas for biological control against pests (Majerus et al., 2006). It is a generalist predator that feeds on a wide range of prey species (Berkvens et al., 2010) such as aphids and many non-Hemiptera species such as Thysanoptera, larvae of Lepidoptera, Coleoptera, Hymenoptera and Diptera (Evans, 2009). Due to its high dispersal capabilities, it has now spread from agricultural areas into adjacent natural habitats (Adriaens et al., 2008; Brown et al., 2011). The invasion by *H. axyridis* has generated many reports of negative impacts to native species (Katsanis et al., 2013). For example, it can outcompete native species for food resources which lead to

native species displacement and disruption of trophic levels (Alhmedi et al., 2010; Katsanis et al 2013).

Harmonia axyridis is one of the dominant members involved in intraguild predation in aphidophagous predator groups (Adriaens et al., 2008). Aphidophagous predator species aggregate in areas with high aphid density, where they form an aphidophagous guild that predominantly preys on aphids (Slogget et al., 2008; Agarwala and Bardhanroy, 1999). *Harmonia axyridis* feeds on other coccinellids (mainly larvae, Majerus et al., 2006)) and predators (Roy and Migeon, 2010) and causes a decline in these native predators (Gardiner and Landis, 2007). The composition of the different species in an area will therefore change in the presence of this beetle due to competition for resources and intraguild predation (Hironori and Katsuhiro, 1997).

Harmonia axyridis is known to occur in different habitats such as natural landscapes, agricultural areas, and green spaces in urban areas (Osawa, 2011; Vandereycken et al., 2012). In agricultural areas, *H. axyridis* often occupy the margins between crop fields and natural vegetation remnants. Here they often wait for an aphid outbreak in the crop fields (Alhmedi et al., 2007) when they move to these fields (Osawa, 2011; Vandereycken et al., 2012; Vandereycken et al., 2013). Aphids are the most preferred prey and determinants of the migration of *Harmonia axyridis* between different areas (Hemptinne et al., 1992). This species can therefore often move long distances to aphid infested areas (Honek et al., 2007), where they can find more food for their survival and reproduction (Slogget and Majerus, 2000). As a biological control agent, *H. axyridis* has been reported to be effective in pest suppression in agricultural areas (Koch et al., 2006). In natural habitats, *H. axyridis* may cause negative effects to native taxa by displacing them from their habitats, or feeding on native species (Gardiner et al., 2009).

Honek, (1982) showed that the communities of coccinellids including *H. axyridis* in most habitats is highly influenced by environmental factors. For example, photoperiod, temperature, food quality, population density and moisture all have effects on these taxa (Rankin and Rankin, 1980). This is largely driven by changes in plant species composition due to seasonal change (especially the

herbaceous component of ecosystems), which eventually cause movements in prey species and therefore the coccinellids that prey on them (Iperti, 1999). *Harmonia axyridis* can also respond to seasonal changes by changing habitats in order to find shelter. For example, during colder seasons they may migrate to protected areas such as buildings where they can overwinter (Berkvens et al., 2010). This invasive species can again move back to feeding sites in spring when temperatures are more suitable (Wheeler and Henry, 1981; Honek et al., 2007).

Harmonia axyridis was first recorded in agricultural areas in Riviersonderend, Western Cape Province, South Africa in 2006 (Stals and Prinsloo, 2007). It has now been recorded in all nine provinces (Brown et al., 2011) and in six biomes of South Africa (Stals, 2010). This species has also been found in different agricultural types such as vegetable crops, vineyards, deciduous and subtropical orchards and forestry plantations (Stals, 2010). The origin of *H. axyridis* in South Africa is still unknown, but Stals, (2010) hypothesised that this invasive species might have arrived through the importation of goods, as there are no records of intentional introduction of this invasive species in South Africa (Nedved et al., 2011). The impact of *H. axyridis* on native South African arthropod communities is unknown.

Globally ecological systems have been negatively impacted by the presence of *H. axyridis*. This study will highlight any negative effects from *H. axyridis* in the urban-agricultural-natural landscape in the Cape Floristic Region, Western Cape, South Africa. This will be valuable as it will allow us to determine if this is a species of concern for biodiversity (within the natural areas), or of agricultural benefit. This information will also be able to let us know the habitat preferences of this invasive species in these landscape features, and how it uses them throughout the year. Ultimately this study will determine the threat status of *H. axyridis* in the Western Cape and if eradication or control is required, then will help us determine the most opportune moments and locations to do this.

1.8. Aims and objectives of this study

The overall aim of this study is to determine how the invasive Harlequin ladybeetle uses the local urban-agricultural landscape in the Western Cape Province, South Africa and to assess its threat to other ladybeetles, predators and herbivores.

The objectives are:

- to investigate the habitat preferences of the invasive Harlequin ladybeetle in terms of agricultural, natural and urban landscapes within the greater Stellenbosch area (Chapter 2);
- to determine its seasonal distribution to ascertain how it uses the landscape during the course of a year (Chapter 2);
- to determine if the Harlequin ladybeetle has a negative effect on native arthropod communities, i.e. predators, herbivores and/or other coccinellids respectively (Chapter 3).

To achieve these objectives I sampled agri-urban landscapes around the greater Stellenbosch area, in urban areas, vineyards, on the edges between natural vegetation and vineyards and deep within natural fynbos vegetation. Sampling was conducted every two months for a year. I determined the habitat preferences of this species per season in Chapter 2. In Chapter 3 I investigated how the abundance of the Harlequin ladybeetle relates to native arthropod communities. This was done for the entire assemblage sampled and for predators, herbivores and other coccinellids separately. I discuss the main results of the research conducted in chapter 4.

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Chapter 2: Spatial and temporal variations of *Harmonia axyridis* across a urban-agricultural landscape mosaic

Abstract

The invasive Harlequin lady beetle, *Harmonia axyridis*, has been introduced as a biological control agent to agricultural areas worldwide. Due to its high mobility and dispersal capabilities, it has now spread from agricultural to natural habitats where it has established. As a result, the species has become an invasive pest of global concern. *Harmonia axyridis* uses different landscape features during different seasons, with food availability and environmental factors dictating these patterns. In this study, I determined how the invasive Harlequin lady beetle uses a local urban-agricultural-natural landscape mosaic in the Western Cape Province, South Africa. I specifically set out to investigate distribution patterns of *H. axyridis* across agricultural (vineyard), natural (fynbos) and urban habitats to determine if this pattern differs seasonally. Sampling was conducted every two months for a year in vineyards, natural habitats, edges between natural habitats and vineyards, and urban areas. Adult *H. axyridis* had higher abundance in urban areas than other habitats, with very few individuals encountered in vineyards. Edge and natural habitats had fairly similar numbers of *H. axyridis*. Larvae were only captured in urban areas. Highest numbers of larvae were recorded during May and July (end autumn and winter), while adults were most abundant during July (winter). Edge and natural habitats showed an increase in adult abundance during May and November (early winter and spring) with very few individuals collected during July. This suggests that urban areas are important breeding grounds and likely also overwintering sites for *H. axyridis* and that adults move from these breeding grounds into natural and edge habitats. This habitat also appears to offer the most control options. The low abundance of *H. axyridis* in vineyards suggests that they do not contribute much in terms of biological control in these habitats.

2.1. Introduction

Alien invasive species are regarded as a threat to new environments if they have the ability to alter and threaten native biodiversity (Raghubashi et al., 2005; Sujay et al., 2010). Some invasive alien species have been introduced for economic gain, however, they have established beyond habitats they were introduced to, and have invaded local natural systems (Roy and Migeon, 2010). This has been compounded by globalization and the trafficking of goods and people around the world (Hulme, 2009) and has facilitated the distribution of alien invasive species worldwide (Meyerson and Mooney, 2007). For example, the Harlequin ladybeetle, *Harmonia axyridis*, originally from Asia (Koch, 2003), now has a global distribution aided by importation of goods (Brown et al, 2008) and as biocontrol agent against agricultural pests (Brown et al., 2011). It has now spread into many natural habitats (Brown et al., 2007).

Alien invasive species are mostly long lived, aggressive generalists that undergo rapid population growth and have high dispersal potential (Sujay et al., 2010). *Harmonia axyridis* has a high fecundity that rapidly increases its population size in newly encountered areas, while its high mobility allows it to rapidly colonise new areas (Labrie et al., 2006). Invasive species are also considered to be one of the biggest threats to biodiversity (Roy et al., 2012). They have the ability to transform the structure and composition of local ecosystems through the displacement of native species directly or indirectly, either by competition for resources or by changing ecosystem function (Sujay et al., 2010). In the case of *H. axyridis*, it outcompetes native species for food resources which may consequently lead to displacement of these native taxa (Katsanis et al., 2013)

Invasive arthropods are one of the largest and most diverse groups of invasive alien species in the world (Roy et al., 2011). Generalist predators, like *H. axyridis*, aggressively feed on a large variety of prey insects (Snyder and Evans, 2006; Evans, 2009), making them prime candidates as biological control agents in agricultural areas (Giliomee, 2011; Roy et al., 2011). It is a very aggressive species that makes it a very successful predator for use in biological control (Cottrell and Yeargan, 1998). Economically they are considered to be a beneficial species as they help to suppress pests, such as

aphids, and so are used globally to increase crop production (Obrycki and Kring, 1998; Roy et al., 2011). Like many other invasive predators, *H. axyridis* therefore has a long history in biological control (Obrycki and Kring, 1998; Snyder and Evans, 2006). However, globally there are many reports of native species decline after the introduction of *H. axyridis* (Colunga-Garcia and Gage, 1998). Their presence could lead to the disruption of the local ecosystem through competition for resources or intraguild predation (Kindlmann et al., 2011), For example, *H. axyridis* feed on variety of pest species and many other non-target species which can lead to competition with other predators for food (Alhmedi et al., 2010). *Harmonia axyridis* has the ability to survive in different habitats and under different climatic conditions while being able to disperse rapidly (Majerus et al., 2006; Soares et al., 2008). Food availability is the most influential factor of migration in coccinellids (Bianchi and van der Werf, 2004) and encourages their high levels of dispersal as they move from patch to patch searching for suitable prey (Sloggett et al., 2008). Aphid density has been found to be one of the most important factors that determine the population density and migration of coccinellids (Honek, 1982; Hemptinne et al., 2000; Sloggett and Majerus, 2000). An increase in consumption on aphids increases their chances of survival, reproduction and larval development (Yasuda and Ishikawa, 1999).

Many other factors other than food availability may also influence the migration of the species across landscape features (e.g. microclimate and season; Iperti, 1999). Rankin and Rankin (1980) showed that, migration in insects may occur as a way of responding to unfavourable conditions, with environmental factors such as photoperiod, temperature, food quality, population density and moisture possible influencing factors. During cold conditions, *H. axyridis* migrate to overwintering sites where they aggregate to create warm microclimates. They hibernate in cracks and crevices in rocks, concrete buildings, caves and even in litter (Labrie et al., 2008; Berkvens et al., 2010). In the northern hemisphere, during spring and summer, both adults and larvae of *H. axyridis* prey on aphids, but when winter approaches they migrate from feeding sites to overwintering sites (Riddick et al., 2000). In agricultural systems, coccinellids often use hedgerows, forest edges, grass tussocks and debris in natural vegetation as overwintering sites, and they also depend on aphids available in those sites for

feeding (Bianchi et al., 2006). Therefore coccinellids may use different landscape features for different reasons, for example feeding, reproduction and overwintering (Woltz and Landis, 2014).

In Africa, *H. axyridis* was intentionally introduced into agricultural areas of Tunisia and Egypt for biological control (Nedved et al., 2011; Brown et al., 2011). In South Africa, *H. axyridis* was first recorded in agricultural areas in 2006, specifically on Tygerhoek Experimental farm, Riviersonderend, Western Cape Province (Stals and Prinloo, 2007). It has now been recorded in agricultural areas, urban areas, six biomes and in all provinces of South Africa (Stals, 2010). It is assumed to have arrived and established in South Africa before 2001, but how the species was introduced remains unknown. Stals (2010) hypothesised that the species might have arrived through the importation of goods into the country, particularly as there are no records of intentional *H. axyridis* introduction into South Africa (Nedved et al., 2011).

In this study, I aim to determine how the invasive Harlequin ladybeetle uses the local landscape mosaic seasonally. I expect to find that *H. axyridis* uses different landscape features for different reasons at different times of year due to prevailing environmental conditions, as it does in the northern hemisphere. These results are important as they will allow us to understand habitat preferences of *H. axyridis* that may allow insight into how best to control it.

2.2. Materials and Methods

2.2.1. Study area and site selection

The Cape Winelands, of the Western Cape Province is part of the greater Cape Floristic Region (CFR) (Bargmann, 2003; Fairbanks et al., 2004), which is considered to be one of the world's richest plant diversity hotspots (Cowling, 1990; Goldblatt and Manning, 2002). The climate of the CFR is Mediterranean, with long hot and dry summers and short, wet winters (Goldblatt, 1997). Four Biomes are recognised in the CFR (Mucina and Rutherford, 2006), of which the Fynbos Biome and the Succulent Karoo biome are the most unique and species-rich (Cowling et al., 1997). The Fynbos

Biome includes three major vegetation types, namely fynbos, renosterveld and strandveld (Mucina and Rutherford, 2006). Fynbos vegetation dominates the region, and is defined by the families Proteaceae, Ericaceae and Restionaceae (Manning, 2007). It is prone to fire and grows on infertile sandy or rocky soils (Linder, 2003; Goldblatt and Manning, 2002). Renosterveld is the second largest vegetation type, dominated by Renosterbos (*Dicerothamnus rhinocerotis* (L. f.) Koekemoer), grasses and seasonally active geophytes, occurring on richer fine-grained soils (Goldblatt and Manning, 2002).

The study was conducted in and around the town of Stellenbosch (33º55′12″S 18º51′36″E) situated in the Cape Winelands district, Paardeberg Mountains (33º35′46.74″S 18º51′07.13″E) in the west coast district and Grabouw (34º09′S 19º01′E) in the Overberg district (Fig. 2.1). These areas are dominated by three landscape elements, namely agriculture (predominately vineyards), urban areas and natural habitats (mostly dominated by Fynbos and Renosterveld) which extends from the borders of vineyards, up to the mountain tops (Mucina and Rutherford, 2006).

Sites were established in each of the three landscape features (vineyards, urban areas and natural areas), as well as on the boundaries between the vineyards and the natural vegetation (edge habitats). Sites on the edge habitats were included as these are often regarded as highly disturbed areas that invasive species frequently invade in. Vineyard sites were located 30 m from the edge and natural sites were located more than 30 m from the natural edge to minimise possible edge effects (Murcia, 1995; Ewers and Didham, 2008). Edge sites were located in the natural vegetation border 5 m from the vineyards border. Urban sites were selected only within the town of Stellenbosch, as this represented the dominant urban area within the study area. Sites were restricted to green areas associated with Oak trees. The vegetation types sampled in urban area were mainly grass, weeds, herbs, flowers and young shrubs that were growing under and around Oak trees. Ten sites were selected for each landscape feature (Natural, Vineyard, Urban and Edge (between vineyard and natural)), giving a total of 40 sites. Sampling for *H. axyridis* was conducted every two months for a period of one year (from July 2013 to May 2014). All 40 sites were sampled within the first week of

each particular month (total of 240 sampling events). Sampling was restricted to sunny and windless days.

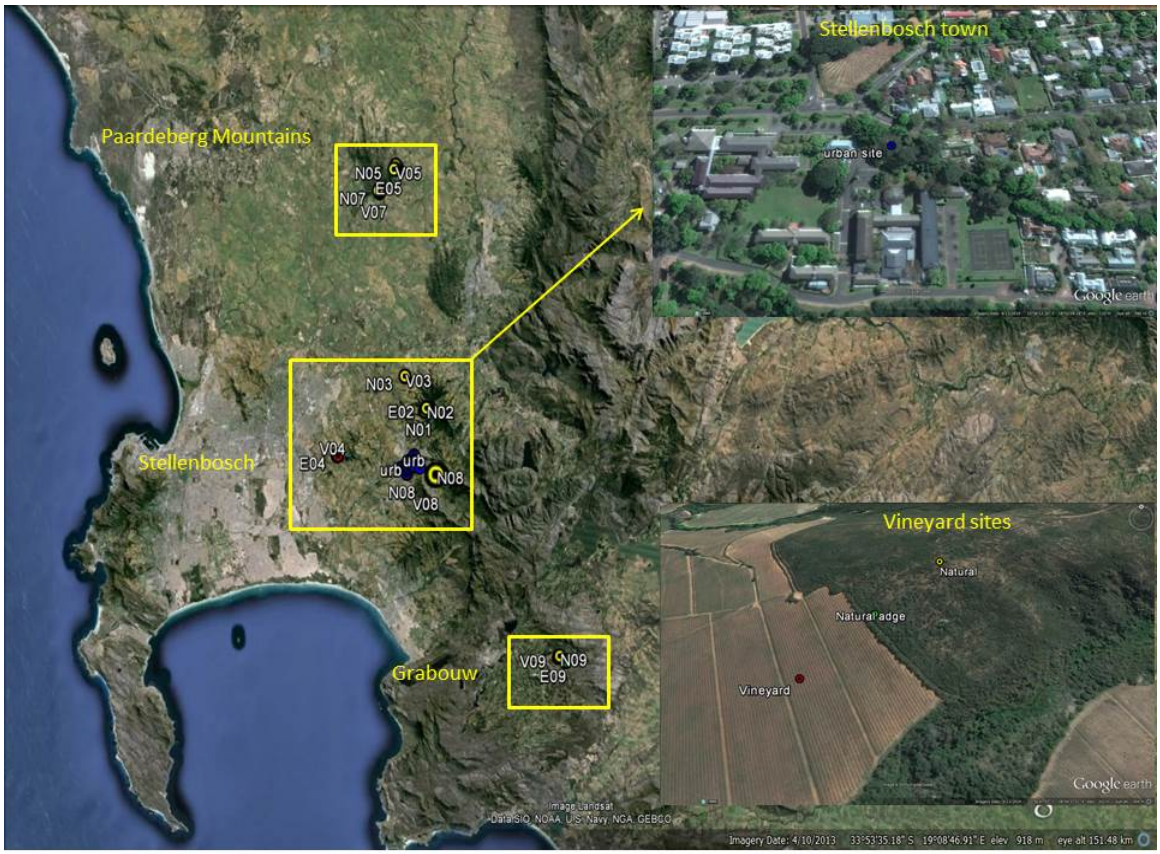

Fig 2.1: Map showing the study sites in Stellenbosch, Paardeberg Mountains and Grabouw, Western Cape, South Africa. All ten urban sites were selected within Stellenbosch. All vineyard, natural edge and natural sites were in agricultural areas in these three towns. Letters and numbers represent sites on the landscape features, V= vineyards, E= edge, N= natural and U= urban. Numbers are 1-10 in each landscape feature

2.2.2. Sampling technique and arthropod curation

Sampling for *H. axyridis* involved the use of a petrol-driven vacuum sampler, a narrow-hosed vacuum sampler (N-type), STIHL SH 86 model (STIHL, Germany). It is a reverse engine machine that draws in air at a high speed (770 m^3/h) through a narrow tube (<15 cm diameter). A bag constructed from fine net was attached to the end of the tube to trap and collect insects. This method is considered ideal

to sample arthropods in the understory vegetation types such as short grass, shrubs and small trees (Leather, 2005).

At each site, the vacuum sampler was used for 10 minutes in which the opening of the tube was directed towards the leaves and branches of plants to collect all foliage living arthropods, while moving at a constant slow pace. Contact with the ground was avoided to prevent collecting soil. Plants were vacuumed from their bases to their tops up to a height of 1.5 meters. In vineyards, both vines and cover crops were sampled. Samples were placed in clear plastic bags and frozen to kill all arthropods before identification. All collected arthropods were separated from leaves and other debris, and the numbers of both adult and larval *H. axyridis* present in each bag were determined. Reference specimens are housed in the Stellenbosch University Entomology Museum (USEC), Stellenbosch, South Africa.

Environmental factors were recorded to investigate their influence on the distribution of *H. axyridis*. These included temperature (maximum and minimum) and rainfall data that was obtained from Stellenbosch University's Sonbesie and Reënmeter permanent weather stations, Stellenbosch. The five day average maximum and minimum temperature, as well as total rainfall for five days prior to each sampling event was used as explanatory environmental variables in statistical analyses.

2.2.3. Data analyses

Generalized Linear Models (GLMs) with Poisson distribution and log-link function were used to compare the abundance of both adult and larval *H. axyridis* between landscape features and between monthly sampling events (O'Hara, 2009; Zuur et al., 2010) in STATISTICA v12 (Statsoft corporation, USA). These models also included as fixed effects maximum temperature (TempMax; the average daily maximum temperature five days prior to sampling), minimum temperature (TempMin; the average daily minimum temperature five days prior to sampling) and rainfall (daily average five days prior to sampling). Further GLZ models were calculated to compare the fixed effects of landscape element, TempMax, TempMin and rainfall for each month and each habitat separately. Posthoc

analyses were performed on significant factors using a Tukey posthoc test in STATISTICA v12 (Statsoft corporation, USA).

2.3. Results

Significantly more adults and larvae of *H. axyridis* were collected in urban areas over the entire sampling period (Fig. 2.2; Table 2.1). In fact, larval *H. axyridis* were solely collected at urban sites. Natural sites had significantly more numbers of *H. axyridis* than edge sites. Vineyards contained very low numbers of this species (Fig. 2.2).

When considering *H. axyridis* numbers collected bi-monthly and for all sites combined, significantly more adults were collected during July (mid-winter) than during any other sampling period (Fig. 2.2) (Table 2.2.). During other sampling periods the numbers of adults had a little significant difference in between except for those collected during March (late summer/early autumn), which were significantly lower than for all other sampling periods (Fig. 2.2). Larval numbers were significantly higher during May (late autumn) and July (mid-winter) and very low during other months. There was a significant difference between larval abundance and sampling months, and temperature (TempMin) (Table. 2.1).

When considering *H. axyridis* numbers collected per sampling event, adults were mostly confined to urban areas in July (mid-winter) and September (spring) (Fig. 2.3). However, during other sampling periods (November (late spring/early summer) – May (late autumn)), the proportion of adults collected from other habitats increased (Fig. 2.3).

The abundance of larval and adult *H. axyridis* were significantly influenced by most variables tested (Table 2.1). Minimum and maximum temperature and rainfall were negatively correlated with adult *H. axyridis* abundance (Table 2.1). Similarly, temperature parameters were negatively associated with larval abundance, but rainfall had no effect on larval abundance (Table 2.1).

Environmental variables (TempMax, TempMin, Rainfall, habitat and months) had an influence in the abundance of adult and larval *H. axyridis* (Table 2.1).

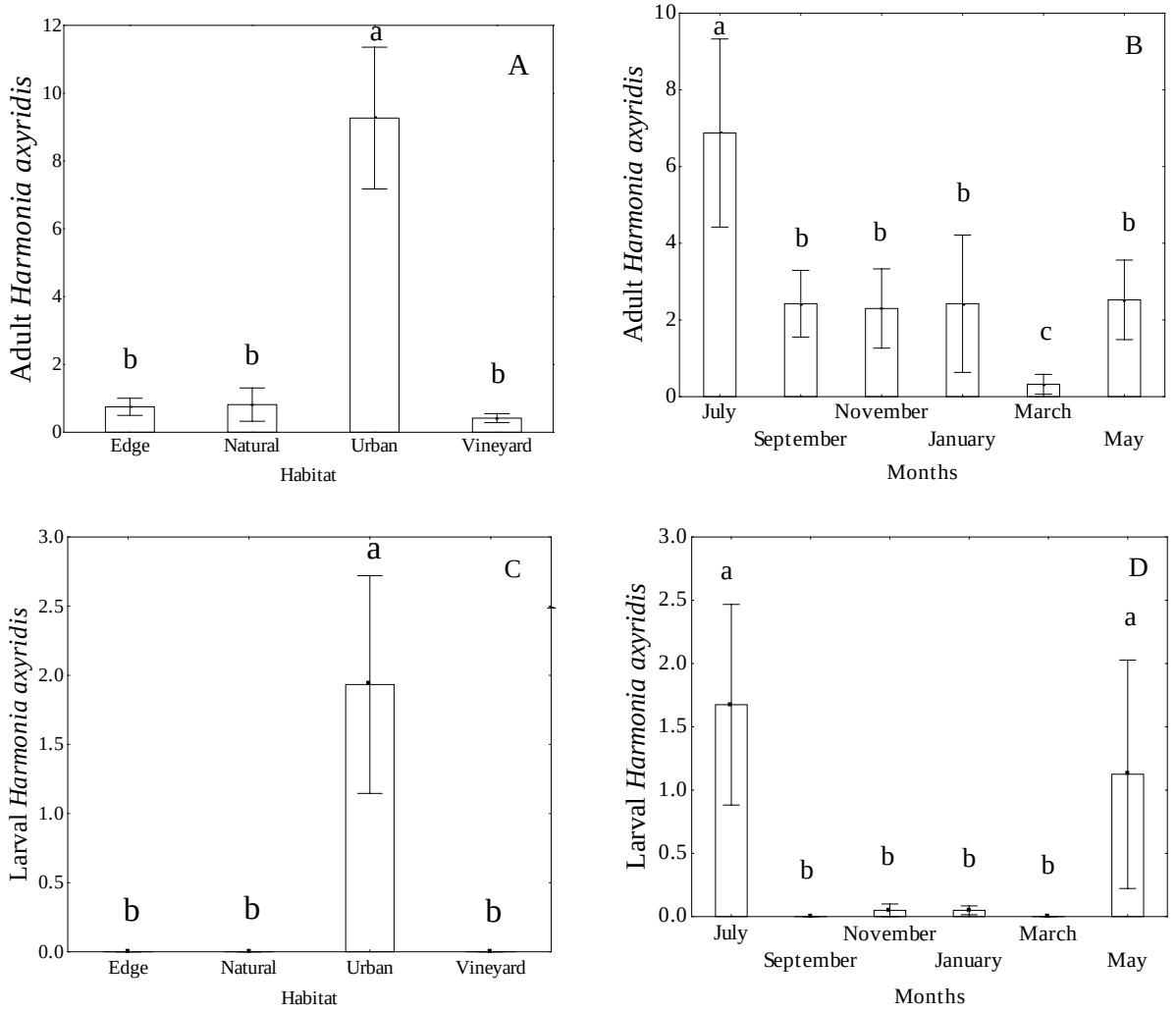

Fig 2.2: Mean abundance (±1 SE) of adult and larval *Harmonia axyridis* collected per habitat type and month sampled. Different letters above bars represent significantly different means (5 % level).

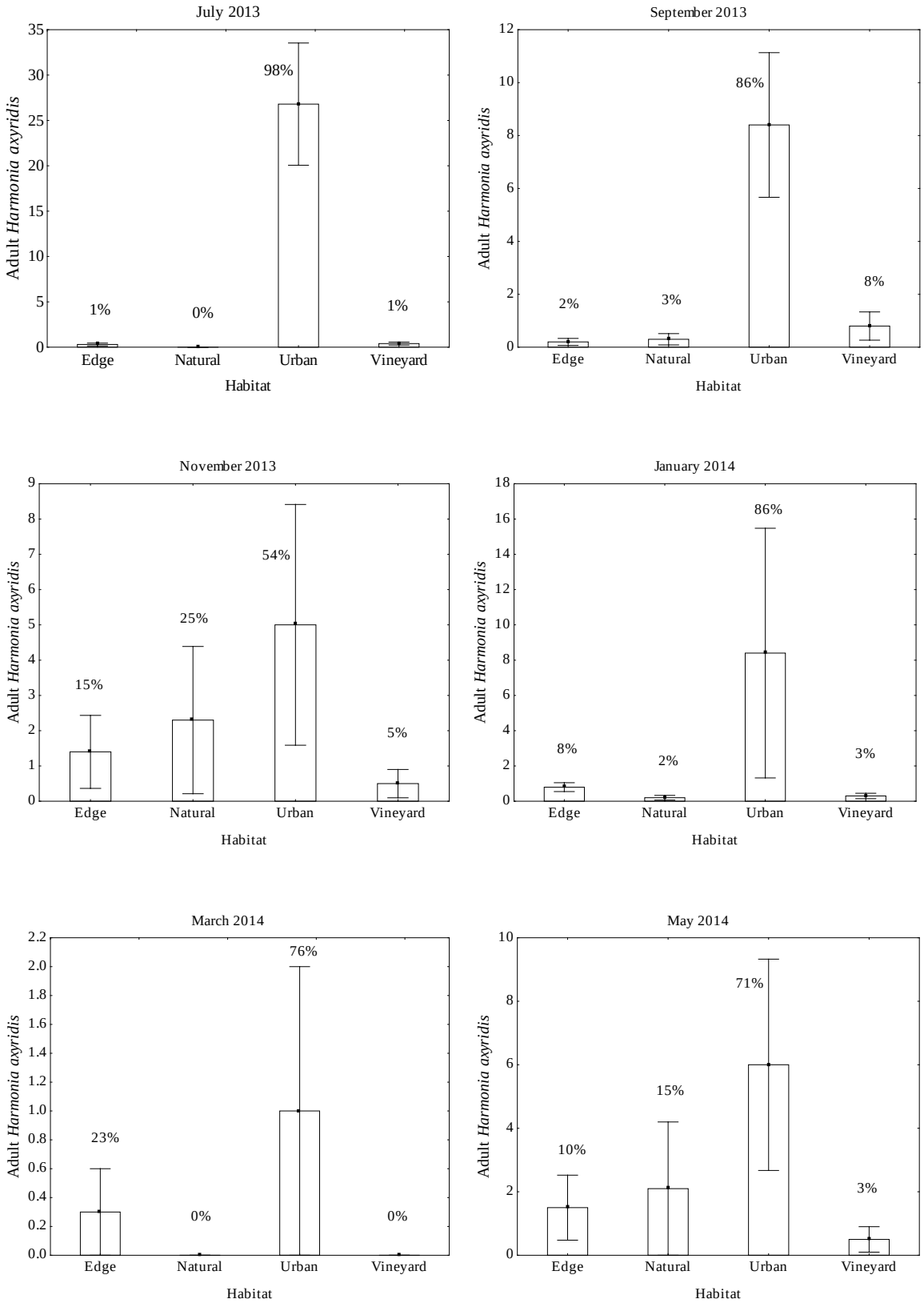

Fig 2.3: Mean abundance (±1 SE) of adult *Harmonia axyridis* per habitat type for each month sampled. Numbers above bars represent the percentage (%) of total *Harmonia axyridis* individuals collected in each habitat for that particular sampling period.

Table 2.1: Results of Generalized linear model (GLZ) analyses with Poisson distribution and log-link function to investigate the relationship between the abundance of *Harmonia axyridis* (adults and larvae) and the environmental variables; habitat type (habitat), sampling period (month), maximum temperature (TempMax), minimum temperature (TempMin) and rainfall (rain).

All seasons	Variables	df	Wald χ^2	p-value
Adult abundance				
	TempMax	1	4.20	0.040
	TempMin	1	8.87	0.003
	Rain	1	4.72	0.030
	Habitat	3	69.52	< 0.001
	Month	5	21.63	0.001
Larval abundance				
	TempMax	1	5.58	0.018
	TempMin	1	29.96	<0.001
	Rain	1	0.71	0.39
	Habitat	2	11.97	0.017
	Month	5	68.81	<0.001

Table 2.2: Results of Generalized linear model (GLZ) analyses with Poisson distribution and log-link function to determine the relationship between adult *Harmonia axyridis* numbers and environmental variables per month sampled. [1]March results were excluded from analyses as only 13 individuals from two sites (urban and edge) were collected.

Months	Variables	df	Wald $\chi 2$	p-value
July 2013	TempMax	1	12.47	<0.001
	TempMin	1	0.24	0.62
	Rain	1	44.81	<0.001
	Habitat	1	4.03	0.04
September 2013	TempMax	1	25.55	<0.001
	TempMin	1	0.05	0.476
	Rain	1	2.94	0.086
	Habitat	3	1.38	0.708
November 2013	TempMax	1	1.14	0.284
	TempMin	1	12.85	<0.001
	Rain	0	-	-
	Habitat	2	0.66	0.717
January 2014	TempMax	1	5.72	0.016
	TempMin	1	1.84	0.174
	Rain	1	0.00	0.939
	Habitat	3	5.13	0.162
[1]May 2014	TempMax	1	1.47	0.224
	TempMin	1	13.19	<0.001
	Rain	1	0.50	0.476
	Habitat	2	0.87	0.646

Table 2.3: Results of generalized linear model (GLZ) analyses with Poisson distribution and log-link function to test the relationship between adult *Harmonia axyridis* numbers and environmental variables per habitat type.

Habitat	Variables	df	Wald stat.	p-value
Edge	TempMax	1	1.01	0.314
	TempMin	1	0.00	0.952
	Rain	1	0.25	0.614
	Month	5	4.80	0.439
Natural	TempMax	1	0.00	0.963
	TempMin	1	2.80	0.093
	Rain	1	0.01	0.915
	Month	2	14.45	0.002
Urban	TempMax	1	14.98	<0.001
	TempMin	1	6.84	0.008
	Rain	1	0.37	0.542
	Month	2	8.41	0.014
Vineyard	TempMax	1	0.62	0.428
	TempMin	1	0.04	0.840
	Rain	1	2.35	0.124
	Month	4	9.98	0.075

2.4. Discussion

Harmonia axyridis was found to be most common in the urban areas, when compared to other landscape features sampled. This preference for urban landscapes might, although not recorded here, be as a result of a high abundance of aphids in these areas, especially during July and May. Yasuda and Ishikawa (1999) showed that high number of aphids trigged reproductive behaviour in urban sites, with adults and larvae present on oak trees and other vegetation. It therefore appears that local weather conditions and the movement of aphids are influencing the movement of this species across

this landscape (Iperti, 1999). Larvae were only found in urban areas, suggesting that urban areas are very important for *H. axyridis* reproduction.

Here, vineyards had a very low abundance of *H. axyridis*, while edge and natural vegetation adjacent to vineyards also showed low abundances. This may be due to the scrubby nature of the native vegetation, with Thomson and Hoffman, (2009), showing that, high abundance of coccinellids are only recorded on the vineyard edges that are adjacent to specific natural vegetation types, in that case wooded vegetation. This is likely to be due to the presence and preferences of natural enemies in the landscape (Bianchi et al., 2013). Ultimately, *H. axyridis* presence or absence is determined by food availability with Elliott et al., (2002) showing that the abundance of coccinellids in crop fields increased with increased abundance of aphids and decrease with aphid scarcity. It is therefore likely that there exists a scarcity of preferred food for this species in vineyards.

Urban sites had highest abundance of *H. axyridis* when compared to the other habitats (edge, natural and vineyards). During November and May *H. axyridis* showed almost the same abundance pattern with high numbers recorded in urban areas, followed by natural sites, edge site and finally vineyards. These were the only months that this invasive insect was collected in large numbers outside urban areas. This suggests that the beetles move out of the urban areas into the vineyards and natural areas seasonally. This is in contrast to other studies where the agricultural patches supported more *H. axyridis* than the surrounding areas (Magagula and Samways, 2001; Nault and Kennedy, 2003; Woltz et al., 2012). Magagula and Samways, (2001), showed that although agricultural areas were the preferred habitat, riparian habitats played a major role in their distribution in savanna. These results support the idea that both wooded vegetation and areas with high levels of moisture are favoured by *H. axyridis*. Furthermore, Nault and Kenedy, (2003), added that agricultural areas with a high abundance of aphids were preferred by coccinellids. Therefore food availability and rainfall (and the interaction of these two elements) influence the movement of *H. axyridis*.

Seasonal usage of habitats is an important consideration for how invasive species use landscapes and their potential risk of spread. Habitat edges showed a comparatively high abundance of this invasive

species in two months, November and May only. Similarly, natural vegetation had a high abundance of this species in November and May, with very low numbers recorded for January and September, and an absence during July and March. Urban areas are used during all months and seem to be the main source population for *H. axyridis*. This agrees with a study by Gardiner et al., (2014) in the USA, where they found a high abundance of coccinellids in urban areas throughout the sampling period. My results confirm that urban areas are used by *H. axyridis* for overwintering and breeding. From here this species shows movement patterns to other landscape features (as also found by Villegas et al. (2013)) such as edge and natural vegetation habitats. This agrees with Purse et al., (2014) that showed that *H. axyridis* occupied urban land cover more than other landscape features, due to buildings used as good hibernation sites and an abundance of tree species that provide foraging sites. Furthermore, urban areas are the only landscape features that showed the presence of larvae of this species. Coccinellid ovipositision sites are associated with high prey density (Aragwala and Bardhanroy, 1999). Although not explicitly tested here, it is likely that aphid numbers are particularly high in the urban environment during winter.

In conclusion, *Harmonia axyridis* is distributed across the entire landscape in this region. Urban areas seem to be the preferred habitat for this species, and the only habitat used as a breeding site. This suggests that if control measures were to be implemented for this species, urban areas would be a good area to target, especially during winter when high numbers of larvae and adults are present. The control of aphids in these urban areas would also likely reduce the numbers of *H. axyridis*. The spread of this species seems likely to be linked to urban environments and planted trees and less so into the natural and agricultural environments.

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Chapter 3: The influence of *Harmonia axyridis* on Western Cape arthropod diversity

Abstract

Harmonia axyridis is considered one of the most successful invasive arthropod predators globally. After entering new ecosystems, it rapidly spreads into different habitats, including natural vegetation. In many European countries, *H. axyridis* has been reported as a threat to native arthropod biodiversity. In this study, I aim to determine the influence of the invasive Harlequin lady beetle on the local fauna in and around Stellenbosch, Western Cape Province, South Africa. I further investigate how this species influences local species across agricultural (vineyards), natural (fynbos) and urban areas at different times of the year. Sampling was conducted every two months for a year in vineyards, natural habitats, edges between natural habitats and vineyards, and urban areas. *H. axyridis* had an impact on native arthropod diversity, and particularly affected functional groups (non-*Harmonia* ladybeetles, predators and herbivores) at different times of the year. There was a significant positive correlation between *H. axyridis* abundance and the abundance of non-*Harmonia* ladybeetles, which suggests that these two groups are responding to the same variables and resources. There was a negative correlation between *H. axyridis* abundance and the abundance of predators. Abundance and richness of the overall arthropod community were negatively affected by the presence of *H. axyridis* for the focal groups (non-*Harmonia* ladybeetles, predators and herbivores). *Harmonia axyridis* appears to disrupt the local arthropod community, competes with predators and negatively influences herbivores. As *H. axyridis* disturbs local arthropod diversity patterns, this study suggests that *H. axyridis* should be controlled in, or preferably removed from these ecosystems.

3.1. Introduction

Invasive alien species are known to cause negative effects to native biodiversity (Elliott et al., 1996; Pejchar and Mooney, 2009), especially as most invasive species are capable of dispersing rapidly into new areas and impact native species (Kindlmann et al., 2011). *Harmonia axyridis* (Coleoptera: Coccinellidae) is such an invasive species. This arthropod predator is native to Asia (Koch, 2003), but has been repeatedly introduced in agricultural areas worldwide for biological control (Brown et al., 2011). Due to its high dispersal behaviour, it is also able to invade different habitats outside agricultural areas (Snyder et al., 2004; Vandereycken et al., 2012).

Many invasive alien predators have been intentionally introduced in agricultural areas for biological control (Rabitsch, 2010), while others have gained entry through human activities (Hulme, 2009). Coccinellids, such as *Harmonia axyridis*, have a long history in biological control to reduce pests in agricultural areas (Alyokhin and Sewell, 2004), and are often considered beneficial in areas where they have accidently been released. Its generalist behaviour and high mobility has made it a highly sought after biological control agent in agricultural areas (Majerus et al., 2006). On the other hand, the high mobility and the aggressive feeding habits make *H. axyridis* a threat to local biodiversity (Katsanis et al., 2013).

Invasive alien species are a threat to native biodiversity (Allerndof and Lundquist, 2003; Didham et al., 2005; Evans et al., 2011), whether they are introduced intentionally or unintentionally (Hoogendoorn and Heimpel, 2002, 2004). Invasive species can cause negative effects such as the decline and local extinction of native species (Gurevitch and Padilla, 2004; Adriaens et al., 2008). For example, since the arrival of *H. axyridis* in many countries, there have been reports of it causing the decline of native species (Alhmedi et al., 2010).

Harmonia axyridis is a generalist predator species that feeds primarily on aphids, although its diet includes a variety of prey species including non-Hemiptera species such as Thysanoptera, larvae of Lepidoptera, Coleoptera, Hymenoptera and Diptera (Evans, 2009). There is evidence that it competes with native species for available resources, which can lead to displacement of native species in natural

habitats (Katsanis et al., 2013). *Harmonia axyridis* can cause further negative impacts through competition, as it can disrupt normal trophic functioning by attacking other predators (Majerus et al., 2006). Invasive predators can also engage in intraguild competition with native predators as they both target the same prey species (Gardiner and Landis, 2007) and *H. axyridis* has been shown to cause a decline in native predators through competition and displacement (Burgio et al., 2002).

In the northern hemisphere *H. axyridis* has been declared a problematic invasive species (Majerus et al., 2006; Brown et al., 2007). In the southern hemisphere, it has been released as biological control agent in South America in Mendoza Province, Argentina, but now it has spread to Brazil (Martins et al., 2009; Brown et al., 2011), and negative impacts on native coccinellids has been reported (Martins et al., 2009). In Africa, it has been released as a biological control in Egypt, Tunisia (Brown et al., 2011) and Kenya (Nedved et al., 2011).

Whether the Harlequin ladybeetle has a negative impact on native arthropods in South Africa is unknown, but the species has been recorded in agricultural areas (Stals and Prinsloo, 2007) and in six different South African biomes (Stals, 2010). In this study I aim to determine whether the invasive Harlequin ladybeetle impacts native arthropod fauna. Here, I wish to ascertain if these effects are limited to specific guilds (predators or herbivores) and if this extends to other coccinelids.

3.2. Materials and Methods

3.2.1. Study area and site selection

The study was conducted in and around the town of Stellenbosch (33º55′12″S 18º51′36″E), Paardeberg Mountains (33º35′46.74″S 18º51′07.13″E) and Grabouw (34º09′S 19º01′E). These are agricultural dominated areas that consists of three landscape elements (agricultural, natural and urban areas), with vineyards the most predominant agriculture type. These districts are situated in the Cape Floristic Region (CFR), Western Cape, South Africa (Fairbanks et al., 2004). The CFR is characterised by a Mediterranean climate, with long hot and dry summers and short, wet winters

(Goldblatt, 1997). The CFR has four recognised biomes (Mucina and Rutherford, 2006) with the Fynbos Biome and the Succulent Karoo biome the most unique and species-rich (Cowling et al., 1997). The Fynbos Biome includes three major vegetation types, namely fynbos, renosterveld and strandveld (Mucina and Rutherford, 2006). The study area is dominated by fynbos vegetation defined by the three plant families Proteaceae, Ericaceae and Restionaceae (Manning, 2007). It is prone to fire and grows on infertile sandy or rocky soils (Linder, 2003; Goldblatt and Manning, 2002). Renosterveld is the second largest vegetation type, dominated by Renosterbos (*Dicerothamnus rhinocerotis* (L. f.) Koekemoer), grasses and seasonally active geophytes, occurring on richer fine-grained soils (Goldblatt and Manning, 2002).

A total of 40 sites were established for this study. Ten replicated sites were established in vineyards, urban, edge in the natural vegetation boundary area adjacent to vineyards, as well as natural areas. Sampling was conducted in July 2013, September 2013, November 2013, January 2014, March 2014 and May 2014, with the actual sampling done in the first week of each month during sunny and windless days. Thus, in total there were 240 sampling events. Sites located in vineyards were more than 30 m from the edge and sites located in natural vegetation were more than 30 m from the edge to overcome edge effects (Murcia, 1995; Ewers and Didham, 2008). Sites on the boundary were located in the natural vegetation edge 5 m from the vineyards border. In urban areas, sites were established in the town of Stellenbosch. They were restricted to green areas associated with oak trees.

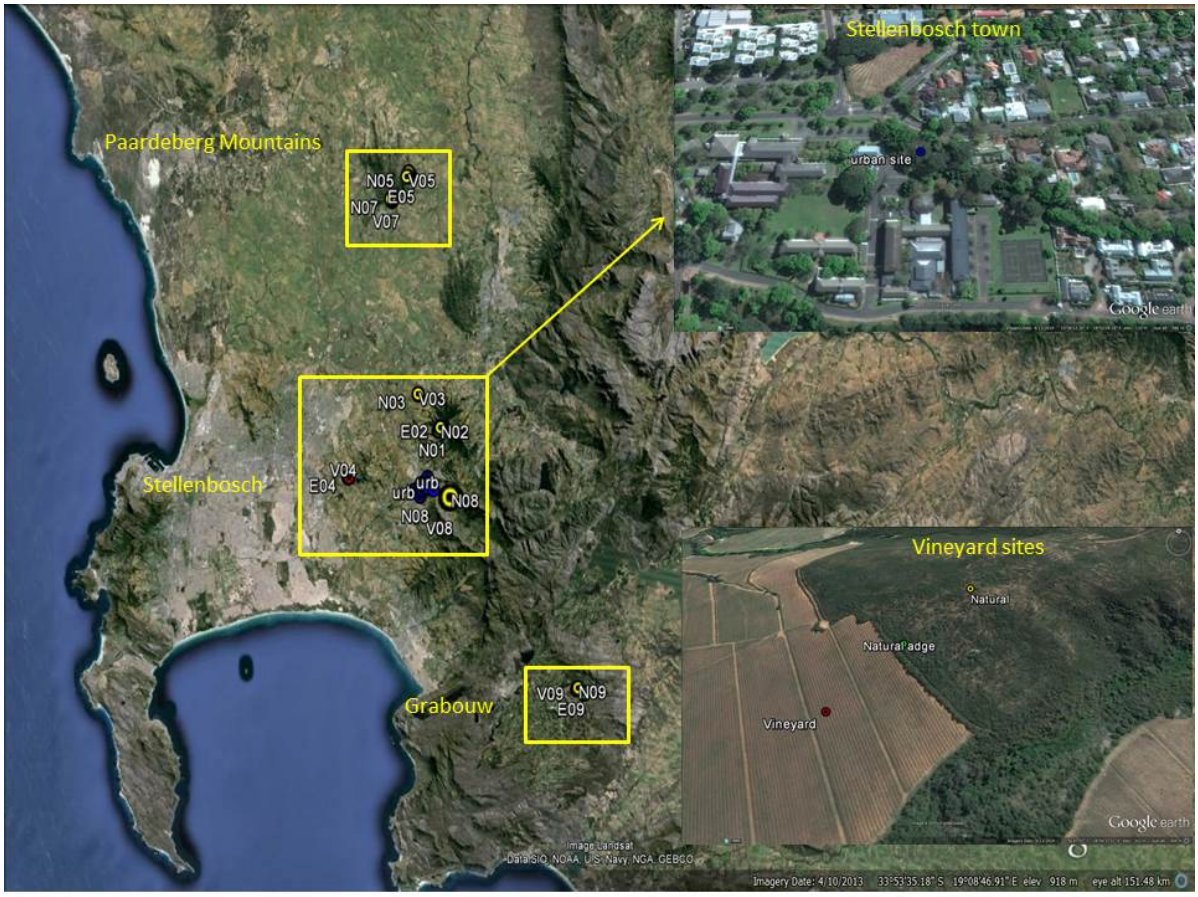

Fig 3.1: Map showing the study sites in Stellenbosch, Paardeberg Mountains and Grabouw, Western Cape, South Africa. All ten urban sites were selected within Stellenbosch town. Vineyard, natural edge and natural sites are all in agricultural areas and were selected around all three towns. Letters and numbers represent sites on the landscape features, V= vineyards, E= edge, N= natural and U= urban. Numbers are 1-10 in each landscape feature

3.2.2. Sampling technique and arthropod curation

All 40 sites were sampled using a narrow-hosed vacuum sampler, STIHL SH 86 (STIHL, Germany). It is a reverse engine machine that draws in air at a high speed (770 m^3/h) through a narrow tube (<15 cm diameter) (Leather, 2005). A bag made of fine netting was attached to the end of the tube and was inserted in the tube to trap and collect insects. This method is ideal to sample insects in understory vegetation types such as short grass, shrubs and small trees (Leather, 2005).

The sampler was used to sample at each site for a period of 10 minutes. The instrument was directed towards the vegetation to collect living arthropods. Only plants of about 1.5 meters high were sampled. In vineyards, both vines and the cover crops were sampled. Samples were placed in clear

bags and then frozen to kill all arthropods. Specimens collected were separated from the leaves and other debris, then grouped into morphospecies, and identified to family level. All morphospecies were placed into one of the following groups: Harlequin ladybeetles, non-*Harmonia* ladybeetles, predators and herbivores. All specimens are housed in the Stellenbosch University Entomology Museum.

Environmental variables for the study area were recorded to investigate their influence on the distribution of *H. axyridis*, overall arthropod community, non-*Harmonia* ladybeetles, predators and herbivores. Temperature (maximum and minimum) and rainfall data was obtained from Stellenbosch University's Sonbesie and Reënmeter permanent weather stations, Stellenbosch. The five day average maximum and minimum temperature, as well as total rainfall for five days prior to each sampling event was used as explanatory environmental variables in statistical analyses.

3.2.3. Data analyses

Generalized Linear Models (GLMs) with negative binomial-log model with a log-link function (where appropriate), and Poisson distribution and log-link function were used to determine the relationship between the invasive Harlequin ladybeetle and native assemblages, namely: non-*Harmonia* ladybeetles, predators and herbivores (O'Hara, 2009; Zuur et al., 2010) in STATISTICA v12 (Statsoft corporation, USA). These models also included maximum temperature (TempMax; the average daily maximum temperature five days prior to sampling), minimum temperature (TempMin; the average daily minimum temperature five days prior to sampling), rainfall (daily average five days prior to sampling) months and habitat as fixed effects.

To investigate whether the invasive Harlequin beetle and environmental variables (months, habitats, temperature and rainfall) affect the composition of native species groups (non-*Harmonia* ladybeetles, predators and herbivores), I used Canonical Correspondence Analysis (CCA) to test the composition of all groups in relation to each variable (Ter Braak and Šmilauer, 2012) in CANOCO 5. Overall arthropod community was tested to see if environmental variables have an effect on them, the same was done to each individual group (non-*Harmonia* ladybeetles, predators and herbivores) to see how their assemblages are influenced by environmental variables across the landscape features.

3.3. Results

The invasive Harlequin ladybeetle did not have a significant impact on the overall species richness and abundance of local arthropods sampled here. However when tested against each group (non-*Harmonia* ladybeetles, predators and herbivores), the abundance of the invasive Harlequin ladybeetle shows a significantly positive correlation with non-*Harmonia* ladybeetle abundance (Fig 3.2; Table 3.1). There is also a significantly negative correlation between the abundance of the Harlequin beetle and predator abundance (Fig 3.2; Table 3.1). There was no impact detected on the abundance of herbivores (Table 3.1).

There was a significant difference in arthropod richness and abundance between months sampled for all groups tested. Habitat sampled also significantly influenced the richness and abundance for all groups (Table 3.1). Maximum temperature showed a significant influence on overall arthropod abundance. On individual groups, only herbivore abundance showed a significant response to maximum temperature. Minimum temperature showed to have an impact on the local overall arthropod abundance and richness. Both the abundance and richness of individual groups (non-*Harmonia* ladybeetles, predators and herbivores) were significantly influenced by minimum temperature (Table 3.1).

Table 3.1: Results of Generalized Linear Model (GLZs) with negative binomial log model with a log-link function, and Poisson distribution with a log-link function was used to calculate a Wald χ^2 value to determine if the Harlequin ladybeetle, months, habitat, minimum and maximum and rainfall affect the abundance and species richness of non-*Harmonia* ladybeetles (nHb), predators (pred), herbivores (herb) and the overall species (SppA and SppR). A= Abundance, R= Richness, HB= Invasive Harlequin beetle.

Variables	SppA	SppR	nHbA	nHbR	predA	predR	herbA	herbR
HB	0.7	0.9	15.1***	2.1	0.5***	0.2	2.5	0.0
Months	39.1***	114.8***	18.0***	12.4*	26.1***	32.9***	53.0***	90.7***
Habitat	17.2***	376.3***	18.6***	11.5*	24.6***	182.3***	18.9***	220.5***
TempMax	0.1*	0.8	0.0	1.2	1.7	1.1	4.4*	0.0
TempMin	9.4*	28.0***	5.2*	1.1	6.7***	12.5***	5.4**	14.7***
Rain	0.1	1.5	0.4	1.04	0.0	1.4	0.4	0.3

*< 0, 05, **<0, 01, ***< 0,001

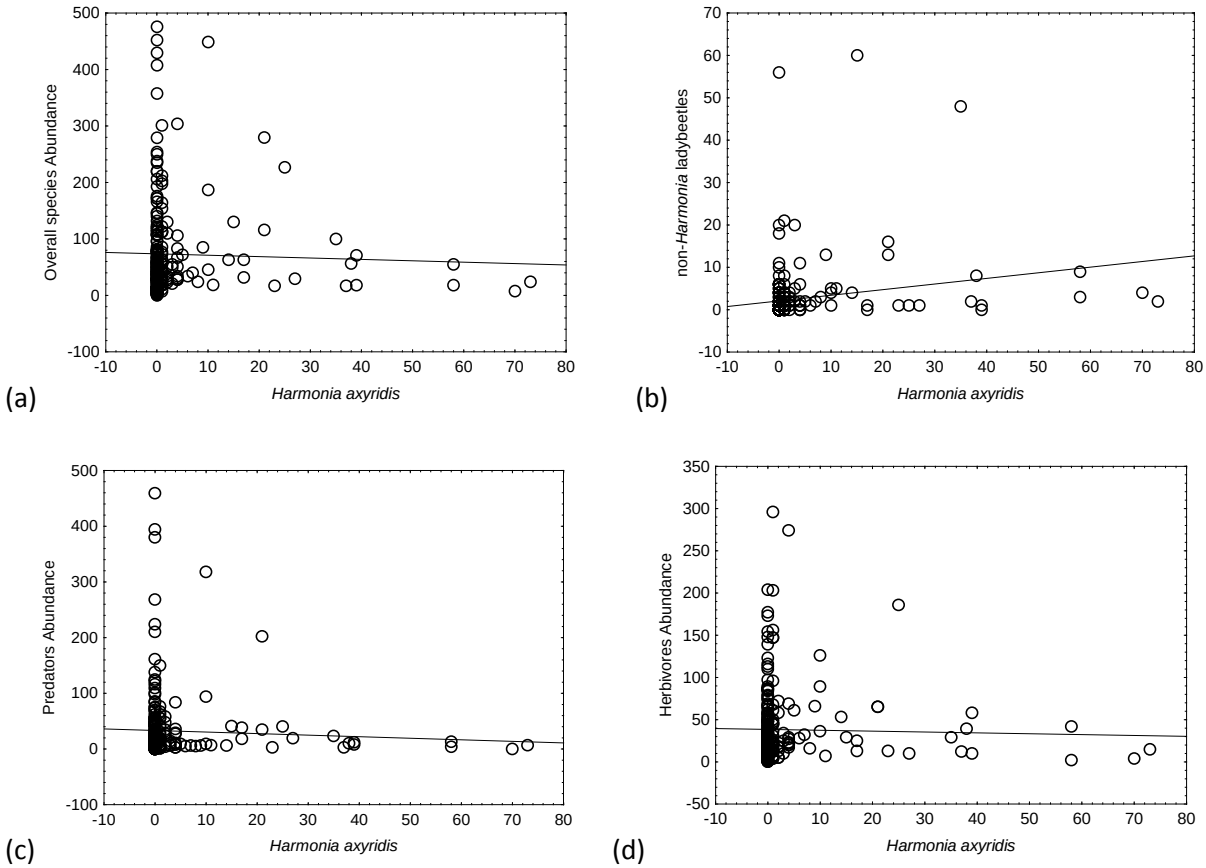

Fig 3.2: Correlations between *Harmonia axyridis* and (a) overall arthropod abundance, (b) non-*Harmonia* ladybeetles, (c) predators and, (d) herbivores

Canonical correspondence analyses (CCA) indicated that overall species composition significantly change in response to all environmental variables, habitats and sampling months (Table 3.2). Adult Harlequin beetle abundance had a significant influence on overall species composition, it also had a significant influence on the composition of other groups (non-*Harmonia* ladybeetles, predators and herbivores), although larval abundance did not (Table 3.2). Non-*Harmonia* ladybeetles showed significant differences in composition in July, January and May, and in all habitats. TempMax, TempMin and rainfall also showed no significant effect on non-*Harmonia* ladybeetles. There was a significant difference between the abundance of non-*Harmonia* ladybeetles and adult *Harmonia axyridis* (Table 3.2). Predators and herbivore assemblages were significantly influenced by sampling months, habitat sampled, as well as temperature and rainfall (Table. 3.2).

Table 3.2: Summary of results of canonical correspondence analyses (CCA), used to test the composition of overall arthropod numbers, non- *Harmonia* ladybeetles (nHb), predators and herbivores in relation to environmental variables: months, habitat, maximum and minimum temperature, rainfall and the invasive Harlequin beetle.

	All species		nHb		Predators		Herbivores	
Variables	Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value	Pseudo-F	p-value
Sampling time								
July	2.5	0.002	3.7	0.006	2.4	0.002	2.4	0.002
September	2.7	0.002	1	0.356	2.4	0.002	2.6	0.002
November	1.6	0.002	0.8	0.576	1.5	0.028	1.6	0.002
January	1.9	0.002	3.6	0.008	1.8	0.002	1.9	0.002
March	1.9	0.002	1.6	0.156	1.9	0.002	1.9	0.002
May	1.9	0.002	3	0.008	2	0.002	1.8	0.002
Habitats								
Vineyard	2.5	0.002	4.3	0.002	2.9	0.002	2.3	0.002
Edge	1.7	0.002	5.6	0.002	1.4	0.006	1.7	0.002
Natural	1.8	0.002	12	0.002	2.1	0.002	1.5	0.002
Urban	2.7	0.002	9.2	0.002	2.4	0.002	2.4	0.002
Environment								
TempMax	2.9	0.002	1.8	0.082	2.7	0.002	2.8	0.002
TempMin	2.5	0.002	1.8	0.09	2.4	0.002	2.5	0.002
Rainfall	2.1	0.002	1.4	0.178	2.5	0.002	1.7	0.002
Invasive species								
Adult *Harmonia*	1.8	0.002	4.9	0.002	1.9	0.01	1.5	0.03
Larval *Harmonia*	1.4	0.172	1.6	0.116	0.9	0.408	1.8	0.04

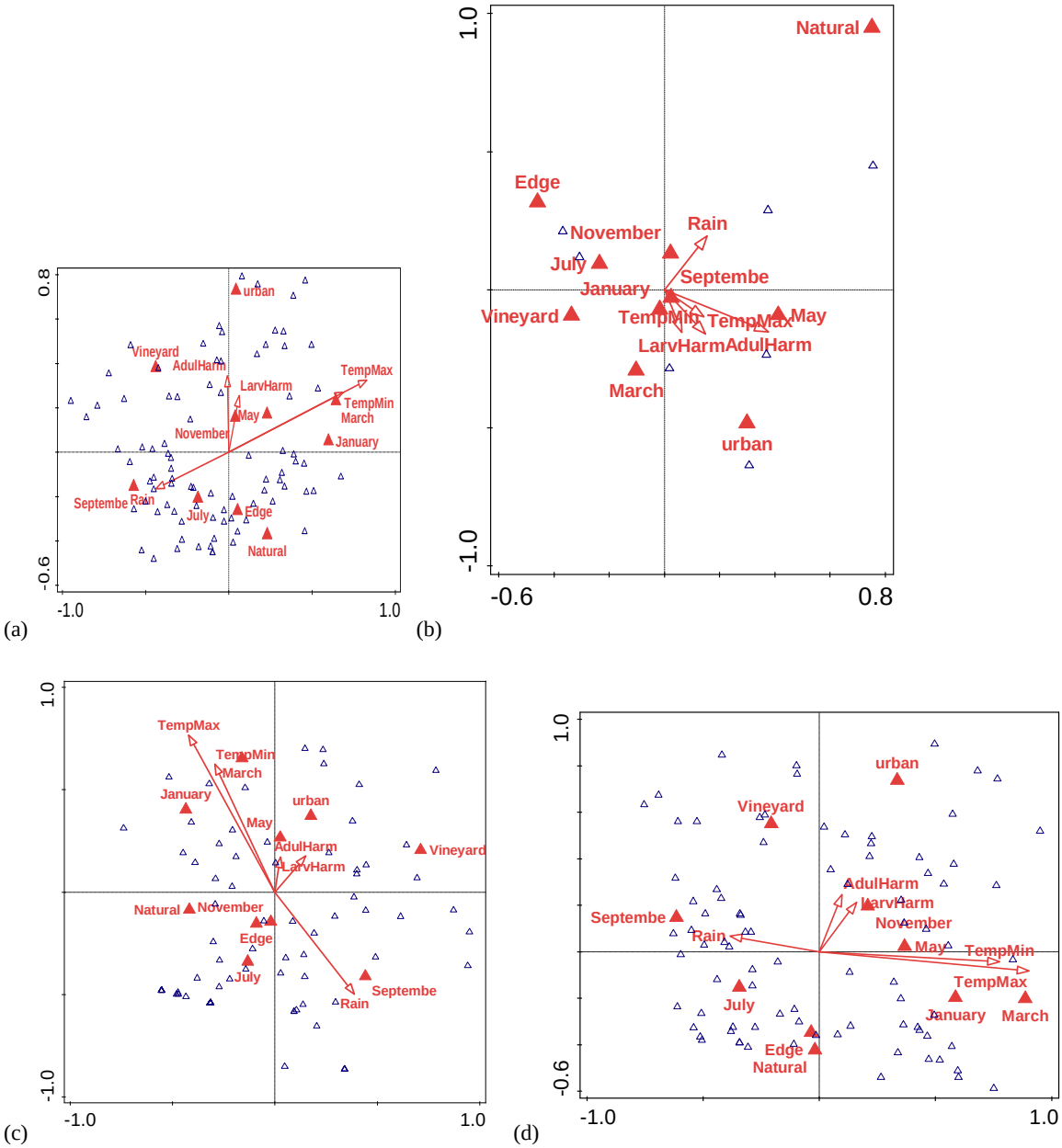

Fig 3.3: Canonical Correspondence Analyses (CCA) for (a) Overall species (b) non-*Harmonia* ladybeetles (c) predators (d) herbivores in all sites during different months and, and the environmental variables of temperature (TempMax and TempMin) and rainfall.

3.4. Discussion

Harmonia axyridis adults had no significant effect on the abundance and richness of the whole arthropod assemblage. Yet, I show here that, *H. axyridis* had significant impact on the abundance of some individual groups such as non-*Harmonia* ladybeetles and predators. The effect of *H. axyridis* on

the abundance of non-*Harmonia* ladybeetles was positive. This indicates that *H. axyridis* and non-*Harmonia* ladybeetles respond to the same environmental variables and resources during this sampling period. These similar resources appear to include them using the same habitats, where they could both be moving to the same hibernation sites and/or feeding areas at the same time. This contrast with Brown et al., (2011) and Michaud (2002) both of which showed that after the establishment of *H. axyridis*, native coccinellids that were dominant in that area were reduced in abundance. Alyokhin and Sewell (2004) on the other hand showed that the establishment of *H. axyridis* and other invasive coccinellids cause a decline in the abundance of native coccinellids, but once these invasive coccinellids had established, there was an increase in native coccinellids. This would suggest that the positive correlation seen here is not that *H. axyridis* does not affect the native coccinellids, rather than those impacts have already happen and those that remain on the landscape are able to tolerate the presence of *H. axyridis.* *Harmonia axyridis* showed a negative effect on the abundance of predators. The presence of *H. axyridis* in habitats that are used by predators could be causing competition between the two groups for resources in those habitats. *Harmonia axyridis* is also known to attack other predators either by eating them or by chasing them away (Brown, 2003). This would allow *H. axyridis* to become the dominant arthropod predator in this landscape. Mizell (2007) showed that, when *H. axyridis* arrived in areas that were dominated by native coccinellids and other predators, both coccinellids and other predators were reduced in abundance.

During all sampling periods, *H. axyridis* had no effect on either abundance and richness of all arthropods. However, there were effects when the abundance and richness of all groups tested here (non-*Harmonia* ladybeetles, predators and herbivores) were analysed separately and in all sampled habitats. This shows that, the presence of *H. axyridis*, regardless of where it is found, seems to cause the arthropod community to change. This is in agreement with Finlayson et al., (2008) who found that, *H. axyridis* and other invasive coccinellids increased in abundance according to sampling period and in different habitats than native coccinellids.

The negative effect of *H. axyridis* on all groups (overall arthropod assemblage, non-*Harmonia* ladybeetles, predators and herbivores) was also supported by the results from the Canonical

Correspondence Analysis (CCA), where *H. axyridis* adults are showing an effect on the composition of all groups (overall arthropods, non-*Harmonia* ladybeetles, predators and herbivores). Larval *H. axyridis* had an effect on the abundance of herbivores only. Therefore, *H. axyridis* has an effect on the composition of overall arthropods assemblages in all areas where they occur as seen in other agricultural and natural areas systems (Mizell, 2007). The effect this beetle has on the abundance of herbivores increase in areas where *H. axyridis* oviposit where larvae are present in high numbers.

Harmonia axyridis has a negative impact on the local arthropod community. As both predators and herbivores are being affected by this invasive species, *H. axyridis* is likely also to affect the services offered by other arthropods such as predators. The justification for retaining these beetles in the landscape, i.e. for the control of agricultural pests they offer, is undone by their negative effect on local predators in this system. As a result this study supports the control or removal of *H. axyridis* across this landscape.

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Chapter 4: General discussion

4.1. *Harmonia axyridis* in urban-agricultural landscapes

Harmonia axyridis is a eurytopic species, as it feeds on many different prey items and is found in many different habitats (Koch, 2003). Once established, *H. axyridis* often invades and transforms the arthropod communities within natural habitats (Vandereycken et al., 2012). The distribution of *H. axyridis* is influenced by food availability and environmental factors (Osawa, 2011). In this study, I found that *H. axyridis* uses different landscape features (vineyards, natural edge, natural and urban areas) at different times of the year for its various developmental needs (Chapter 2). This was exemplified by the significant difference in the abundance of *H. axyridis* in the different landscape features over seasons. The abundance of *H. axyridis* was here shown to be primarily influenced by prevailing weather conditions. This agrees with Patel and Patel, (2014) who conducted their study in the agricultural area in India, looking at the movement and abundance of *Coccinella septumpunctata* L. (Coleoptera- Coccinellidae) in relation to meteorological factors during different seasons. In addition, their study focused on the agricultural area only. They showed that meteorological factors (maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, sunshine hours, wind velocity and rainfall) had a significant effect on the movement and abundance of *Coccinella septumpunctata* L. (Coleoptera- Coccinellidae). Even though their study was conducted testing a different predatory invasive coccinellid (*Coccinella septumpunctata*), both studies showed that environmental factors influences the movement and abundance of these two predatory invasive species.

In all landscape features (vineyard, natural edge, natural and urban) that were evaluated in this study, most *H. axyridis* individuals were collected in urban areas. This agrees with Purse et al., (2014) who conducted their study in Britain, where they assess the invasion of *H. axyridis* in relation to landscape features (natural habitat and urban area) and climate (rainfall and sunshine). The natural habitat types included coniferous woodland, broadleaf woodland, natural grassland and urban areas. *Harmonia axyridis* had higher abundance in urban areas compared to other landscape features. They concluded

that this is likely the result of urban areas providing ample shelter for overwintering and that these areas contain a high abundance of prey species. Furthermore, the occurrence of lime (*Tilia* sp.) and sycamore (*Acer pseudoplatanus*) increased the abundance of *H. axyridis* as they were preferred foraging sites. The urban sites used in my study were selected around Stellenbosch's greenways containing numerous oak trees, which are heavily colonised by aphids during May and July (Dixon, 1998), which coincides with the cold wet winter season in the Western Cape. Even though it was not specifically tested in this study, the high number of *H. axyridis* in these areas suggests that these aphids likely attract the beetles to urban areas during winter. This is supported by the high abundance of larval *H. axyridis* exclusively collected in urban areas. Similar results were obtained by Osawa, (2000) in a Botanical garden (~10 000m^2 area) in Japan, where he chose different plant species in different sites to test which plants are occupied by *H. axyridis* in relation to aphid density. He found that *H. axyridis* migrate to these plant species in response to prey (aphids) availability. Furthermore, the duration of stay of *H. axyridis* on plants depended on the quality and quantity of available prey. Therefore, *Harmonia axyridis* can utilise different habitats in heterogeneous landscapes following preferred food availability (Osawa, 2000). This was also the case in a study by Vandereycken et al., (2015) in Belgium. The study was conducted on four agricultural crops (broad bean, corn, potato and wheat) and showed that *H. axyridis* aggregates in sites with high quantity prey.

The low abundance of *H. axyridis* in agricultural areas in this study is in contrast to a study by Nault and Kennedy, (2003) in eastern North Carolina, USA. Their study was conducted in different agricultural crops (wheat, potato, corn and soybean), the predominant crops in that area. Kajita and Evans, (2010), In USA, working on Alfalfa, also found that *H. axyridis* had higher abundance in agricultural areas, and the beetles also used these areas as breeding sites. The difference between these studies and the present study is that, those studies were conducted on agricultural crops that are different from the ones here, and that may well be good breeding grounds for aphids, the preferred food for *H. axyridis*.

The high abundance of adult *H. axyridis* in urban areas during winter also indicates that, this invasive species likely move to urban areas to overwinter. Adults were often seen in large numbers on walls of

buildings and on fences. These structures are therefore likely used as hibernating sites. This agrees with a study by Wang et al., (2011) in the rural villages of northeast China, a native area for *H. axyridis*. Their study was conducted in agricultural areas (crop and rice) and the natural vegetation in the surrounding areas characterised by woody vegetation. They found that *H. axyridis* migrate from agricultural areas to urban areas during autumn to find protection on buildings to hibernate when cold conditions are prevalent.

In general, natural vegetation is not often occupied by *H. axyridis*. I found an increase in numbers of adult *H. axyridis* in natural and edge habitats during November, indicating that these beetles probably move to these areas from hibernation/breeding sites (Chapter 2). This is supported by results that indicate larval *H. axyridis* are not found in these habitats. The increase in numbers of adult *H. axyridis* in natural vegetation could be influenced by the specific vegetation type of a particular area or by early season arthropod prey species availability. It is therefore possible that natural fynbos vegetation is not a particularly good vegetation type for this species to feed or breed in. This contrasts with a study by Leather et al., (1999) in England, who showed that the abundance of coccinellids (both invasive and native) in natural habitats of certain vegetation types were high (in that case grasslands).

As agricultural areas are subjected to constant disturbance by management practices, it is possible that the low abundance of *H. axyridis* in vineyards could be caused by some management practices (Freemark and Boutin, 1995; Brown and Schmitt, 2001). This may include the use of chemicals to control pests (Obrycki and Kring, 1998). If successful to control pests, chemical control should reduce food availability for *H. axyridis* in the vineyards. Also, if these pesticides have non-target effects, a reduction in numbers of *H. axyridis* in these areas can be expected. These factors will force *H. axyridis* to move to other habitats to search for prey species.

The most important result of this research is that, as the abundance of *H. axyridis* in vineyards is extremely low, these beetles are not useful as biological control agents in these systems in the Western Cape. The fact that they also breed and hibernate in urban areas suggests that these areas should be targeted for the control of these insects.

4.2. Impact of *Harmonia axyridis* on native species

Harmonia axyridis had a negative impact on arthropod assemblages in the Western Cape (Chapter 3). This beetle seemed to affect the abundance of non-*Harmonia* ladybeetles and predators the most (Chapter 3). *Harmonia axyridis* numbers was positively correlated to the abundance of non-*Harmonia* ladybeetles, suggesting that they coexist in this landscape. This disagrees with Bahlai et al., (2014), Snyder and Clevenger, (2004) and Moser and Obrycki, (2009) who found that the invasion of *H. axyridis* in an area that was dominated by native coccinelids had a negative effect on their abundance. The coexistence between *H. axyridis* and non-*Harmonia* ladybeetles could be influenced by the same preferences (e.g. food and habitat) that these two groups have. As *H. axyridis* has probably been in South Africa longer than currently expected and are well established in the study area, the negative effect seen by other authors could have already taken place, and only those species that are able to co-exist with *H. axyridis* remain. Thus they could be responding to the same environmental variables at the same time, sharing feeding grounds and hibernating sites, while those species unable to tolerate *H. axyridis* have gone locally extinct. The strong negative correlation between the number of *H. axyridis* and the abundance of predators may indicate that *H. axyridis* poses a threat to local predators, and that these may be displacing them. This could be the result of either direct attack on predators or indirectly through competition due to limited availability of prey resources (Ingles and De Clercq, 2011; Alhmedi et al., 2010).

Some impact of *H. axyridis* on the abundance and richness of the overall arthropod communities was clearly detected during each sampling period throughout the year (Chapter 3). The effect on the abundance and richness the focal groups (overall arthropods, non-*Harmonia* ladybeetles, predators and herbivores) was also detected for all habitats. The presence of *H. axyridis* caused a shift in the arthropod community assemblage (Chapter 3). Larval *H. axyridis* also affected herbivore composition (Chapter 3). This indicates that, in areas where *H. axyridis* breed, larvae also prey on certain specific herbivores and therefore alters these communities. This is in agreement with Cottrell, (2004) who showed that larval *H. axyridis* feed on the other native species at oviposition sites, including the eggs of other coccinellids. Results of this study therefore mimic that of Mizell, (2007) who found that the

establishment of *H. axyridis* in an area that was dominated by many predators and herbivores, causes a decline in the abundance of both resulting in *H. axyridis* becoming the dominant predator in the area. This will have a negative impact to the functional ecosystem services provided by arthropods in these systems.

4.3. Main conclusions

In conclusion, *Harmonia axyridis* occurs in different agricultural landscape features in the Western Cape, South Africa. The occurrence of this species seems to be greatly influenced by environmental variables and food availability. Urban areas are the most preferred habitat, especially during July and May. This invasive species moves to other landscape features during late November, but *H. axyridis* is present in the urban areas throughout the year. Vineyards house very low numbers of *H. axyridis*, indicating that this is the least preferred habitat and that *H. axyridis* is a poor biological control agent in vineyards, at least in the Western Cape.

Harmonia axyridis negatively impacts the local arthropod community and could be detected in all sampled landscape features. They therefore likely disrupt functional ecosystem services provided by local biodiversity. As *H. axyridis* negatively affects biodiversity, and has little value as biocontrol agents in agriculture, I recommend that a control programme should be implemented to control this problematic species. Indications from this project suggest that control in the urban areas during winter, when the species is aggregating and there are larvae present, would be the best time and place for control. What would be of interest is to determine how the *H. axyridis* population would react to removal from urban areas and if it also leads to decreased numbers in other landscape features. Control mechanisms should therefore be accompanied by rigorous monitoring in all landscape features to assess if it is effective across the landscape and to ensure that the *H. axyridis* does not shift its breeding sites to other areas.

4.4. References

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